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☐ **1:** Osteoarthritis Cartilage. 2000 Nov;8(6):434-43. Related Articles, Links

Differential effects of SB 242235, a selective p38 mitogen-activated protein kinase inhibitor, on IL-1 treated bovine and human cartilage/chondrocyte cultures.

Badger AM, Roshak AK, Cook MN, Newman-Tarr TM, Swift BA, Carlson K, Connor JR, Lee JC, Gowen M, Lark MW, Kumar S.

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The p38 MAP kinase inhibitor, SB 242235, was evaluated for its effects on the metabolism of bovine and human cartilage and primary chondrocyte cultures. SB 242235 had no effect on proteoglycan synthesis (PG) in bovine articular cartilage explants (BAC), as measured by [(35)S]-sulfate incorporation into glycosaminoglycans (GAGs). In addition, the compound had no effect on IL-1 alpha-induced GAG release from these cultures. However, there was a potent, dose-dependent inhibition of nitric oxide (NO) release from IL-1 alpha-stimulated BAC with an IC(50)of approximately 0.6 microM, with similar effects observed in primary chondrocytes. The effect on BAC was time dependent, and mechanistically did not appear to be the result of inhibition of protein kinase C (PKC), protein kinase A (PKA) or MEK-1. The effect on NO release in bovine chondrocytes was at the level of inducible nitric oxide synthase (iNOS) gene expression, which was inhibited at similar concentrations as nitrite production. In primary human chondrocytes, IL-1 beta induction of p38 MAP kinase was inhibited by SB 242235 with an IC(50)of approximately 1 microM. Surprisingly, however, treatment of IL-beta-stimulated human cartilage or chondrocytes with SB 242235 did not inhibit either NO production or the induction of iNOS. On the other hand, the natural product hymenialdisine (HYM), a protein tyrosine kinase (PTK) inhibitor, inhibited NO production and iNOS in both species. In contrast to the differential control of iNOS, PGE(2)was inhibited by SB 242235 in both IL-1-stimulated bovine and human chondrocyte cultures. These studies indicate that there are species differences in the control of iNOS by p38 inhibitors and also that different pathways may control IL-1-induced proteoglycan breakdown and NO production. Copyright 2000 OsteoArthritis Research Society International.

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1: Regul Pept. 2005 Mar 30;126(3):223-31. Related Articles, Links

ELSEVIER FULL-TEXT ARTICLE

Regulation of corticotropin-releasing factor receptor type 2beta mRNA by mitogen-activated protein kinases in aortic smooth muscle cells.

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The actions of the corticotropin-releasing factor (CRF) family of peptides are mediated by the seven transmembrane-domain G-protein-coupled receptors, the CRF receptors. CRF receptor type 2beta (CRFR2beta) messenger RNA (mRNA) is expressed primarily in the cardiovascular system, where its levels are decreased by urocortin 1 (Ucn1), a novel peptide in the CRF family. In a previous study, we reported that CRFR2beta mRNA levels were partially down-regulated via the cAMP-protein kinase A pathway. This study focused on the involvement of the intracellular mitogen-activated protein (MAP) kinase pathway in the modulation of CRFR2beta mRNA levels. Ribonuclease protection assays showed that decreases in CRFR2beta mRNA levels induced by Ucn1 and cAMP were attenuated by the p38 MAP kinase inhibitor SB202190 or SB203580. This finding suggested that the p38 MAP kinase pathway was involved in this regulation. Anisomycin, a classic p38 kinase activator, increased CRFR2beta mRNA levels in A7r5 cells. This effect of anisomycin was completely reversed by H7, a serine/threonine kinase inhibitor, while both p38 kinase and MAP kinase kinase inhibitors failed to block the increase in CRFR2beta mRNA levels caused by anisomycin. As anisomycin can activate Jun amino terminal kinases, as well as p38 MAP kinase, it is possible that other MAP kinases, such as Jun amino terminal kinases, also contribute to the increase in gene levels. Alternatively, anisomycin may increase CRFR2beta mRNA levels indirectly as a consequence of blocking protein synthesis.

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1: J Pharmacol Exp Ther. 2001 Jul;298(1):331-8. Related Articles, Links

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Cytosolic phospholipase A2 activation by the p38 kinase inhibitor SB203580 in rabbit aortic smooth muscle cells.

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SB203580 [4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1H-imidazole] is widely used as a specific inhibitor of p38 mitogen-activated protein kinase (MAPK). Here we report that SB203580, which blocked p38 kinase activation elicited by anisomycin, increased the phosphorylation and activity of cytosolic phospholipase A2 (cPLA2) and arachidonic acid (AA) release in quiescent vascular smooth muscle cells from rabbit aortae. SB203580 also increased the activity of calcium (Ca2+)/camodulin-dependent kinase II (CaMKII) and ERK1/2 MAPK. The increase in CaMKII activity and cPLA2 phosphorylation caused by SB203580 was attenuated by CaMKII inhibitor KN-93, indicating involvement of CaMKII in cPLA2 phosphorylation by this compound. Since KN-93 also inhibited SB203580-induced ERK1/2 activation, it appears that ERK1/2 activation is also mediated by CaMKII. SB203580-induced cPLA2 phosphorylation was inhibited by depletion of Ca2+ from the medium, by the voltage-operated Ca2+ channel blocker nifedipine, and by the calmodulin inhibitor W-7. cPLA2 translocation from cytoplasm to the nuclear envelope caused by SB203580 was also inhibited in the absence of extracellular Ca2+. Other p38 kinase inhibitors, SB202190 and PD169316, failed to alter CaMKII, ERK1/2, and cPLA2 activity or cPLA2 translocation to the nuclear envelope. These data suggest that SB203580 not only inhibits p38 kinase activity but also increases Ca2+ influx through voltage-sensitive Ca2+ channels, which promotes cPLA2 translocation to the nuclear envelope, and by interacting with calmodulin, activates CaMKII and cPLA2 and releases AA.

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☐ 1: J Cell Physiol. 2004 Mar;198(3):399-407.

Related Articles, Links

ERK1/2 and JNKs, but not p38 kinase, are involved in reactive oxygen species-mediated induction of osteopontin gene expression by angiotensin II and interleukin-1beta in adult rat cardiac fibroblasts.

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Osteopontin (OPN), also called cytokine Eta-1, expressed in the myocardium co-incident with heart failure plays an important role in post myocardial infarction (MI) remodeling by promoting collagen synthesis and accumulation. Angiotensin II (Ang II) and inflammatory cytokines are increased in the heart following MI. We studied the involvement of mitogen-activated protein kinases (ERK1/2, JNKs, p38 kinase) and reactive oxygen species (ROS) in Ang II- and cytokine-induced OPN gene expression in adult rat cardiac fibroblasts. Ang II alone increased OPN mRNA (3.3 +/- 0.3-folds; $P < 0.05$; $n = 7$), while interleukin-1beta (IL-1beta), tumor necrosis factor (TNF-alpha), and interferon-gamma (IFN-gamma) had no effect. A combination of Ang II with IL-1beta or TNF-alpha, not IFN-gamma, increased OPN mRNA more than Ang II alone. Nitric oxide donor, S-nitrosoacetylpenicillamine (SNAP), alone or in combination with Ang II had no effect. Diphenylene iodonium (DPI), inhibitor of NAD(P)H oxidase, and tiron, superoxide scavenger, inhibited Ang II- and Ang II+ IL-1beta-stimulated increases in OPN mRNA. Ang II activated ERK1/2 within 5 min of treatment, not JNKs. IL-1beta activated ERK1/2 and JNKs within 15 min of treatment. A combination of Ang II and IL-1beta activated ERK1/2 within 5 min of treatment. None of these stimuli activated p38 kinase. DPI almost completely inhibited Ang II + IL-1beta-stimulated activation of ERK1/2, while partially inhibiting JNKs. PD98059, ERK1/2 pathway inhibitor, and SP600125, JNKs inhibitor, partially inhibited Ang II + IL-1beta-stimulated increases in OPN mRNA. A combination of PD98059 and SP600125 almost completely inhibited Ang II + IL-1beta-stimulated increases in OPN mRNA. Thus, Ang II alone increases OPN expression, while IL-1beta and TNF-alpha act synergistically with Ang II to increase OPN mRNA possibly via NO independent mechanisms. The synergistic increase in OPN mRNA involves ROS-mediated activation of ERK1/2 and JNKs, not P38 kinase, pathways in cardiac fibroblasts. Copyright 2003 Wiley-Liss, Inc.

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The yin and yang of tumor necrosis factor inhibitors

ABSTRACT

Tumor necrosis factor (TNF) inhibitors have proven highly effective against a number of autoimmune diseases but have been disappointing in treating others. An increase in the risk of *Mycobacterium tuberculosis* and other opportunistic infections has been noted in patients treated with these agents. If we use these drugs, we need to weigh their beneficial and adverse effects.

KEY POINTS

TNF blockers have proven highly effective against rheumatoid arthritis, Crohn disease, psoriasis, and ankylosing spondylitis.

Despite hopes based on theoretical considerations, TNF blockers are not effective against multiple sclerosis, sarcoidosis, Sjögren disease, and congestive heart failure.

Patients using TNF blockers have increased rates of *M tuberculosis* infection and other uncommon opportunistic infections.

TNF blockers are expensive, yet they are highly effective, and they can greatly improve quality of life and perhaps allow patients to avoid future joint replacement and reconstructive surgery.

AS MOST EXPERIENCED PHYSICIANS know, there are two sides to almost any treatment. The most potent and effective therapies are sometimes accompanied by the most profound and dangerous side effects.

The tumor necrosis factor (TNF) inhibitors are a case in point. Since their introduction less than a decade ago, they have revolutionized our approach to a variety of autoimmune disorders, such as rheumatoid arthritis, Crohn disease, ankylosing spondylitis, and psoriasis. The exuberance over these drugs has led researchers to examine TNF inhibitors in areas such as heart failure and endometriosis.

But as the yin and yang of taoist philosophy tell us, the universe is composed of opposites, all contained within a whole. And so it is with TNF inhibitors. Despite their efficacy, rare but formidable toxicities such as lymphoma and infections have occurred. Autoimmune diseases that researchers thought would respond to these drugs have not responded, and in some cases the disease got worse. And the high cost of these genetically engineered drugs can stretch the budget of patients, insurers, and the health system as a whole.

This presentation provides an introduction to the immune system and the role of cytokines, discusses drug design of TNF-blocking agents to target autoimmune diseases, and describes the successes, limitations, and adverse effects of the new therapeutics thus far.

TNF: THE MODEL CYTOKINE

Cytokines are small, nonantibody protein molecules that act as chemical messengers between cells to regulate diverse physiologic processes, including cell growth, differentia-

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*The author has indicated that he has served as a consultant to the Abbott and Amgen corporations.

tion, inflammation, repair, and immunity. More than 150 have now been identified, most of them glycoproteins.

Cytokines were previously referred to by their presumed source, eg, lymphokines from lymphocytes and monokines from monocytes, but since many cytokines, particularly TNF, are produced by a wide range of somatic cells, the more general term cytokine is now used.

Discovery and development of TNF

In the 1970s, Carswell and colleagues[1] were the first to isolate a factor from mouse macrophages that caused experimental tumor death (necrosis). Soon after, the cytokine TNF was identified, sequenced, and cloned.

Other researchers independently "discovered" TNF as they searched for different substances in their field of interest. In 1985, Beutler et al[2] studied a factor, which turned out to be TNF, that caused widespread wasting by acting on lipoprotein lipase and other metabolic pathways. Simultaneously, Dayer et al,[3] searching for a factor that mediated shock, isolated TNF from cells of monocytic lineage.

Shared immunologic pathways

TNF acts on blood-vessel endothelial cells, synovial cells, nervous system pathways, and bone

Effector cells, mast cells, neutrophils, and monocytes engage in an integrated but very redundant immune response. An antigen-presenting cell presents an antigen to certain T cells, which generate a cell-mediated immune response. The T-cell response can be characterized by two important pathways: T helper (Th)1, responsible for granuloma formation and cellular immunity; and Th2, which facilitates humoral immunity and atopy.

Dysregulation of the Th1 responses is involved in rheumatoid arthritis, inflammatory bowel disease, sarcoidosis, psoriasis, and others. The Th2 type responses are responsible for asthmatic allergic diseases, bronchopulmonary aspergillosis, and parasitic diseases. It is a particular challenge to develop specific therapies for these seemingly disparate diseases that share pathways. The goal is to have specific targeted pathway inhibition.

The inflammatory response involves a plethora of cytokines with opposing actions. Some are inflammatory (especially the Th1 cytokines, which include interferon gamma, IL-1, and TNF), and others are anti-inflammatory.

TNF features and effects

TNF is only one of a family of structurally defined molecules. Within the TNF family, more than 20 peptides have been identified. Some, such as CD-40 ligand, lymphotoxin, and FAS-ligand, mediate T and B lymphocyte activation and control apoptosis. Some are starting to be used as therapeutic targets. Their structural similarities have added to the impetus to design specific drugs.

TNF affects many organs. Normally, it is present in nanomolar concentrations and is believed to be essential for tumor surveillance, the regulation of inflammatory response, and perhaps local tissue repair.

Large amounts of TNF are released in response to a lipopolysaccharide challenge. A few hours after a mouse is sensitized with just a few micrograms of lipopolysaccharide, it develops a fever, a disrupted sleep cycle, and an acute response-phase leukocytosis. With moderate concentrations of lipopolysaccharide, inflammatory cytokines can be detected. A large lipopolysaccharide challenge stimulates an out-of-control inflammatory response, leading to multiple organ dysfunction, septic shock, and death.

TNF has a variety of actions. In blood vessels and smooth muscle cells, it promotes proliferation and influences endothelial cells to go from an anticoagulant state to a procoagulant state. In synovium, it promotes proliferation and upregulates adhesion molecules, causing an influx of inflammatory cells. In the central nervous system it disregulates pathways, causing fever and sleep disruption. In bone, it is involved in destruction and repair. TNF affects repair and scar formation by its action on fibroblasts. It also causes direct lysis of tumor cells.

TNF expression

TNF is expressed by a variety of immune cells but is also rapidly induced in many nonimune cells by a variety of stimuli.

TNF is expressed within cell membranes as a homotrimer, with aggregated monomers. These are cleaved by TNF-alpha converting enzyme (a metalloproteinase), allowing TNF molecules to circulate freely and to bind to receptors for TNF on a variety of target cells. These soluble receptors may be cleaved off of

TABLE 1

Indications for TNF blockers

DISEASE	ETANERCEPT	INFLIXIMAB	ADALIMUMAB
Rheumatoid arthritis	Yes	Yes	Yes
Psoriatic arthritis	Yes	Yes	Yes
Ankylosing spondylitis	Yes	Yes	Under investigation
Juvenile rheumatoid arthritis	Yes	Under investigation	Under investigation
Crohn disease	No	Yes	Under investigation
Ulcerative colitis	No	Yes	Under investigation

cell surfaces and decrease the circulating levels of TNF, a process that helps keep the potent TNF system in check.

■ DRUGS DESIGNED TO ALLEVIATE INFLAMMATION

TNF's effects are mediated by two distinct types of TNF receptors, both of which are involved in inflammation and the induction of cell death. TNF and other cytokines interact with their specific receptors, thereby initiating an inflammatory signal.

One drug-design strategy is to neutralize cytokines by developing TNF-specific monoclonal antibodies. Another specific strategy is to use solubilized receptors to bind the circulating cytokine.

Three drugs that inhibit TNF have been approved by the US Food and Drug Administration. Their structural differences confer distinct properties[4]:

- **Etanercept** (Enbrel) is a construct of the Fc portion of immunoglobulin G (IgG) bound to a TNF receptor. It is given by subcutaneous injection and has a half-life of 4.8 days.
- **Infliximab** (Remicade) is a chimeric monoclonal antibody with a human IgG Fc region and murine antigen-binding regions that are highly specific for TNF. It is given by intravenous infusion and has a half-life of 9.5 days.
- **Adalimumab** (Humira) is similar to infliximab, but is wholly of human construct. However, under certain circumstances it still may be perceived by the body as foreign. It is given by subcutaneous injection and has a half-life of 12 to 14 days.

TNF inhibitors can be viewed as a model for biologic therapy for autoimmune inflammatory diseases. More agents with different targets are being investigated, including drugs that modulate IL-2, IL-4, or IL-10.

■ RHEUMATOID ARTHRITIS AS A MODEL

Rheumatoid arthritis is a multisystem inflammatory disease, targeting joints, that occurs in genetically susceptible people. It involves a variety of cells, including T cells, B cells, and accessory cells. Judging by the success of new cytokine-blocking agents, cytokines appear to drive the rheumatoid process. Patients today no longer suffer the highly destructive and deforming effects of the disease common just 25 years ago, and we are now able to set our sights on achieving remission and even disease regression.

In rheumatoid arthritis, methotrexate plus a TNF blocker is better than methotrexate alone

Most drugs used to treat rheumatoid arthritis were originally intended for other diseases, such as tuberculosis and malaria. In the 1990s, Ravinder Maini (who was knighted for his work), Marc Feldmann, and others recognized that cultured synovial tissue contained a "soup" of inflammatory cytokines. They eventually focused their research on two of them: interleukin 1 and TNF.

Although methotrexate is still considered to be the gold standard treatment of rheumatoid arthritis, adding etanercept leads to significantly more gains than are achieved by treating with either medication alone. In the Anti-TNF Trial in Rheumatoid Arthritis With Concomitant Therapy (ATTRACT), Smolen et al[5] found that patients treated with a combination of methotrexate and infliximab had a dramatic slowing of joint damage

as seen by radiography vs patients on methotrexate alone, with healing seen at the highest doses studied (infliximab 10 mg/kg every 4 weeks).[5]

With nearly 7 years of experience with TNF inhibitors, we now know that long-term therapy is feasible. Therapy maintains long-term suppression of disease activity, particularly in rheumatoid arthritis, and there is no evidence of mounting toxicity as is seen with glucocorticoids.

■ OTHER USES FOR TNF BLOCKERS

Crohn disease

Biologic therapy, including TNF inhibitors, is revolutionizing the treatment of Crohn disease and possibly ulcerative colitis as well. Like rheumatoid arthritis, Crohn disease occurs in genetically susceptible people. The disease involves nonspecific granulomatous inflammation, leading to tissue injury and repair.[6–8] Van Dullemen et al[9] showed that a single infusion of infliximab leads to profound healing of the mucosa of patients with active Crohn disease as seen by colonoscopy. Long-term regimens have been approved and are increasingly used.

TNF inhibitors do not work in all diseases in which, in theory, they should

Psoriasis

Biologic treatments including etanercept and infliximab have been used to treat psoriasis. Adalimumab is expected to be approved soon.

All TNF inhibitors appear to be highly effective in treating both psoriasis and psoriatic arthritis and represent major advances in the treatment of these disorders.[10]

Ankylosing spondylitis

Drug manufacturers have traditionally regarded ankylosing spondylitis as a low priority, since the disease is uncommon and causes such severe damage and joint ankylosis that substantial improvement appeared unlikely. However, the effects of TNF inhibitors on the disease have been even more dramatic than for rheumatoid arthritis.[11]

■ THE DOWNSIDE TO TNF INHIBITION

Despite the success of TNF inhibitors, much of the early exuberance is giving way to a more measured view of these drugs. They continue to transform our care of many patients with autoimmune disease, but as you can see from the discussion below, researchers are finding that these drugs do not work in all diseases in which, theoretically, they should. And as experience with these drugs grows, we are seeing some predictable and unexpected side effects. Also, as with all genetically engineered drugs, there is the issue of cost.

■ UNSUCCESSFUL USES OF TNF INHIBITORS

Wegener granulomatosis

TNF blockers showed great promise in the treatment of Wegener granulomatosis in a 6-month open-label study of etanercept in 20 patients.[12] The mean vasculitis activity score for the disease improved dramatically in treated patients, and there were no apparent adverse effects. However, a subsequent multicenter, randomized, placebo-controlled trial[13] in 180 patients followed for a mean of 27 months did not show that etanercept was effective for maintaining remission. Also, the rate of treatment-related complications was high and included a possibly increased rate of solid cancers in the treatment group, who also received cyclophosphamide.

Multiple sclerosis

Multiple sclerosis would appear to be an ideal target for TNF inhibitors: TNF kills oligodendrocytes and injures myelin and is found in plaques and cerebrospinal fluid, correlating with multiple sclerosis activity. Furthermore, animal models of multiple sclerosis showed that disease worsens when TNF activity is enhanced and improves when TNF is downregulated. However, in two small trials, TNF inhibitors were not successful for the treatment of multiple sclerosis, and some treated patients actually had a worsening of their condition. Another disturbing finding is a rare illness resembling multiple sclerosis that has been increasingly reported in patients treated with TNF inhibitors for other conditions.[14]

Other conditions

Etanercept, the first and mostly widely tested TNF blocker, has been unsuccessful for treating Crohn disease, sarcoidosis, Sjögren disease, and congestive heart failure.

■ EVIDENCE OF INFECTION RISKS

TNF serves a homeostatic role. Although high levels of TNF contribute to chronic local or systemic inflammation and joint destruction, low levels increase the risk of infection. The goal of therapy with TNF blockers is not to eliminate TNF completely, but rather to restore a balance of TNF.

The lack of evidence of excess infections in patients treated with TNF blockers during phase III clinical trials reassured the medical community that these drugs were relatively safe in this regard. However, evidence of disturbing trends of increased infections and other diseases is starting to emerge as the use of TNF blockers increases. For example, I had one patient with severe rheumatoid disease uncontrolled with methotrexate and prednisone. We treated her with a 6-week course of infliximab infusions, and 2 weeks later she developed staphylococcal bacteremia and meningitis. She recovered, then developed skin lesions due to *Mycobacterium chelonae*.

Tuberculosis and TNF

TNF is a macrophage activator and appears to be critical in helping the body form granulomas. The granuloma response is critical to protect against disseminated *M tuberculosis* infection. Excessive and prolonged inhibition of TNF signaling leads to exacerbation of tuberculosis. "TNF-knockout" mice develop uncontrolled mycobacterial infections that lead to death.[15,16] *Mycobacterium* also proliferates in wild-type mice treated with anti-TNF antibody.

Gomez-Reino et al[17] analyzed a database of patients in Spain who were treated with infliximab or etanercept and found more than 20 times the incidence of tuberculosis over the background rate, though the risk was markedly greater for infliximab than for etanercept. In the United States, no increase in the incidence of tuberculosis has been detected in patients taking TNF inhibitors, but this may be due to a much lower background rate and less opportunity for disease spread than exists in Spain. Infections from other intracellular pathogens, however, are being reported in patients taking etanercept and especially infliximab, including *Listeria monocytogenes*, *Histoplasma capsulatum*, and others.[18–20]

Test for tuberculosis before starting anti-TNF drugs. All patients being considered for anti-TNF therapy should be screened for tuberculosis exposure . This should include questioning to determine risk factors, testing with purified protein derivative (PPD), and chest radiography. The PPD testing sensitivity varies with the cutoff value used: use a lower cutoff for patients deemed to be at higher risk of tuberculosis infection. Treatment with isoniazid for 9 months is recommended for patients with a PPD test result greater than 5 mm. Anti-TNF therapy should be delayed until isoniazid therapy is completed.

Other potential major adverse effects of TNF inhibition

- An increased risk of non-Hodgkin lymphoma has also been observed in patients taking TNF inhibitors, but patients with moderate to high rheumatoid disease activity have an up to 26 times greater risk of lymphoma,[21–27] making it difficult to determine whether treatment with TNF constitutes an independent risk factor.
- TNF inhibition may increase the risk of atypical neurologic damage, eg, demyelination.
- Recently, TNF inhibitors have been linked to activation of latent viruses, including hepatitis B, which has led to some deaths.
- TNF inhibitors have been linked to vasculitic syndromes.

Disturbing trends of increased infections with TNF blockers are emerging

■ THERAPY COSTLY, BUT BENEFICIAL

Most TNF inhibitors cost about $1,000 per month, which means they are a luxury treatment for many patients. Still, they are an important option for patients with rheumatoid arthritis, Crohn disease, psoriasis, and ankylosing spondylitis. Although expensive, TNF inhibitors often allow patients to return to work, have fewer hospitalizations, and avoid joint replacements and reconstructive surgery.

TNF inhibitors are not yet uniformly accessible, even within the United States. Outside of western countries, they are not available at all. ■

REFERENCES

1. Carswell EA, Old LJ, Kassell RL, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci USA 1975; 72:3666–3670.
2. Beutler B, Greenwald D, Hulmes JD, et al. Identity of tumour necrosis factor and the macrophage-secreted factor cachectin. Nature 1985; 316:552–554.
3. Dayer J-M, Beutler B, Cerami A. Cachectin/tumor necrosis factor stimulates collagenase and prostaglandin E2 production by human synovial cells and dermal fibroblasts. J Exp Med 1985; 162:2163–2168.
4. Calabrese L. Molecular differences in anticytokine therapies. Clin Exp Rheumatol 2003; 21:241–248.
5. Smolen JS, Han C, Bala M, et al. The ATTRACT Study Group. Evidence of radiographic benefit of treatment with infliximab plus methotrexate in rheumatoid arthritis patients who had no clinical improvement: a detailed subanalysis of data from the anti-tumor necrosis factor trial in rheumatoid arthritis with concomitant therapy study. Arthritis Rheum 2005; 52:1020–1030.
6. Gately MK, Renzetti LM, Magram J, et al. The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. Annu Rev Immunol 1998; 16:495–521.
7. Podolsky DK. Inflammatory bowel disease. N Engl J Med 2002; 347:417–429.
8. Murch SH, Braegger CP, Walker-Smith JA, MacDonald TT. Location of tumour necrosis factor alpha by immunohistochemistry in chronic inflammatory bowel disease. Gut 1993; 34:1705–1709.
9. van Dullemen HM, van Deventer SJ, Hommes DW, et al. Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2). Gastroenterology 1995; 109:129–135.
10. Weinberg JM, Bottino CJ, Lindholm J, Buchholz R. Biologic therapy for psoriasis: an update on the tumor necrosis factor inhibitors infliximab, etanercept, and adalimumab, and the T-cell-targeted therapies efalizumab and alefacept. J Drugs Dermatol 2005; 4:544–55.
11. Braun J, Baraliakos X, Brandt J, Sieper J. Therapy of ankylosing spondylitis. Part II: biological therapies in the spondyloarthritides. Scand J Rheumatol 2005; 34:178–190.
12. Stone JH, Uhlfelder ML, Hellmann DB, Crook S, Bedocs NM, Hoffman GS. Etanercept combined with conventional treatment in Wegener's granulomatosis: a six-month open-label trial to evaluate safety. Arthritis Rheum 2001; 44:1149–1154.
13. Wegener's Granulomatosis Etanercept Trial (WGET) Research Group. Etanercept plus standard therapy for Wegener's granulomatosis. N Engl J Med 2005; 352:351–361.
14. Mohan N, Edwards ET, Cupps TR, et al. Demyelination occurring during anti-tumor necrosis factor alpha therapy for inflammatory arthritides. Arthritis Rheum 2001; 44:2862–2869.
15. Flynn JL, Goldstein MM, Chan J, et al. Tumor necrosis factor-alpha is required in the protective immune response against *Mycobacterium tuberculosis* in mice. Immunity 1995; 2:561–572.
16. Mohan VP, Scanga CA, Yu K, et al. Effects of tumor necrosis factor alpha on host immune response in chronic persistent tuberculosis: possible role for limiting pathology. Infect Immun 2001; 69:1847–1855.
17. Gomez-Reino JJ, Carmona L, Valverde VR, Mola EM, Montero MD; BIOBADASER Group. Treatment of rheumatoid arthritis with tumor necrosis factor inhibitors may predispose to significant increase in tuberculosis risk: a multicenter active-surveillance report. Arthritis Rheum 2003; 48:2122–2127.
18. Slifman NR, Gershon SK, Lee JH, Edwards ET, Braun MM. Listeria monocytogenes infection as a complication of treatment with tumor necrosis factor alpha-neutralizing agents. Arthritis Rheum 2003; 48:319–324.
19. Lee JH, Slifman NR, Gershon SK, et al. Life-threatening histoplasmosis complicating immunotherapy with tumor necrosis factor alpha antagonists infliximab and etanercept. Arthritis Rheum 2002; 46:2565–2570.
20. Wallis RS, Broder M, Wong J, Lee A, Hoq L. Reactivation of latent granulomatous infections by infliximab. Clin Infect Dis 2005; 41(suppl 3):S194–S198.
21. Beauparlant P, Papp K, Haraoui B. The incidence of cancer associated with the treatment of rheumatoid arthritis. Semin Arthritis Rheum 1999; 29:148–158.
22. Isomaki HA, Hakulinen T, Joutsenlahti U. Excess risk of lymphomas, leukemia and myeloma in patients with rheumatoid arthritis. J Chronic Dis 1978; 31:691–696.
23. Gridley G, McLaughlin JK, Ekbom A, et al. Incidence of cancer among patients with rheumatoid arthritis. J Natl Cancer Inst 1993; 85:307–311.
24. Thomas E, Brewster DH, Black RJ, Macfarlane GJ. Risk of malignancy among patients with rheumatic conditions. Int J Cancer 2000; 88:497–502.
25. Baecklund E, Ekbom A, Sparen P, Feltelius N, Klareskog L. Disease activity and risk of lymphoma in patients with rheumatoid arthritis: nested case-control study. BMJ 1998; 317:180–181.
26. Mellemkjaer L, Linet MS, Gridley G, Frisch M, Moller H, Olsen JH. Rheumatoid arthritis and cancer risk. Eur J Cancer 1996; 32A:1753–1757.
27. Matteson EL, Hickey AR, Maguire L, Tilson HH, Urowitz MB. Occurrence of neoplasia in patients with rheumatoid arthritis enrolled in a DMARD Registry. Rheumatoid Arthritis Azathioprine Registry Steering Committee. J Rheumatol 1991; 18:809–814.

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Omega-carboxypyridyl substituted ureas as Raf kinase inhibitors: SAR of the amide substituent

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Abstract—Bis-aryl ureas have been disclosed previously as a potent class of Raf kinase inhibitors. Modifications in the amide portion led to an improvement in aqueous solubility, an important characteristic for an oral drug. Based on this finding, we hypothesize that this portion of the molecule is directed towards the solvent in Raf-1.

Most of the known small molecule protein kinase inhibitors bind to a highly conserved ATP-binding pocket and in general are flat, aromatic molecules that mimic the adenine portion of ATP.[1] Therefore, relatively poor drug-like properties such as high logP and low aqueous solubility are major challenges. For example, the introduction of water-solubilizing groups to the 6- and 7-positions of the quinazoline EGFR inhibitors was an important step towards the optimization of their physico-chemical properties.[2]

We have previously reported our focus on Raf-1 kinase as a validated target for the treatment of cancer.[3] The lead compound **1** [*N*-(5-*tert*-butyl-3-isoxazolyl)-*N'*-(4-phenoxyphenyl)urea, Fig. 1] was identified by screening of a combinatorial chemistry library, and exhibits an IC_{50} value of 1100 nM against recombinant human Raf-1 kinase.[4] Optimization of **1** led to a series of potent, orally active Raf-1 kinase inhibitors,[5–8] and culminated in the identification of a clinical candidate BAY 43-9006 (**5**).[3,5]

Introduction of an *N*-methyl carboxamide at the *meta* position of the distal phenyl ring (**2**) increased activity by almost 10-fold.[5] Replacement of the distal phenyl ring with a 4-pyridyl ring (**3**) also significantly improved the potency. Combination of these two structural features leads to highly potent ureas, such as **4**. An additional optimization effort where the isoxazole ring of **4** is replaced by other five-membered heterocycles as well as appropriately substituted phenyl groups led to a clinical candidate BAY 43-9006 **5**.[3,5]

We sought to identify a site for the introduction of water solubilizing groups without affecting the Raf-1 inhibitory potency. In this article, we wish to report our study of the structure–activity relationships of the amide portion of the molecule, directed towards optimizing aqueous solubility.[10]

Our group has previously disclosed synthetic routes to ureas similar to those described in this report, such as **1–5**.[7,8] Furthermore, the general preparation of ureas **26–27** and **37–39** is depicted in Figure 2. Reaction of 5-hydroxynicotinic acid methyl ester with 1-fluoro-4-nitrobenzene, followed by reduction of the nitro group, urea formation, hydrolysis of the ester and subsequent amide formation provides access to amide analogues.

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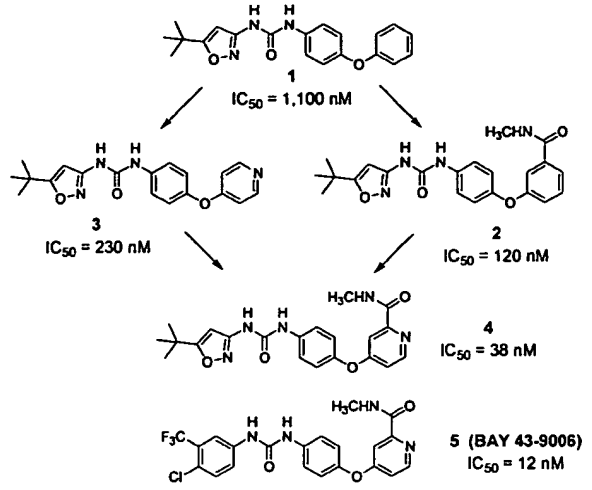


Figure 1. Urea-based Raf-1 kinase inhibitors.

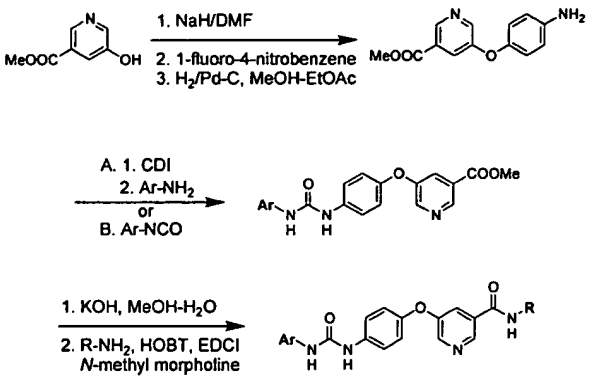
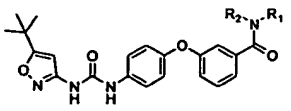


Figure 2. Preparation of ureas **26–27** and **37–39**.

Initial evaluation of the effect of amide substituents larger than methyl revealed no improvement, but demonstrated the existence of an extra space in this binding region of Raf-1 kinase (Table 1). For example, replacement of the *N*-methyl group of **2** with larger alkyl groups such as ethyl or *n*-propyl led to similar Raf-1 potencies[9] (entries **6** and **7**). In contrast, modification of the primary amide group to a secondary amide as in urea **8** was detrimental to the potency, suggesting the importance of a hydrogen-bond donor in this part of the molecule. Increasing the size of the *N*-substituent to benzyl or phenyl led to a less dramatic loss in potency (Table 1, entries **9** and **10**). Similar trends were observed with urea derivatives where the isoxazole heterocycle was replaced by a substituted phenyl group (Tables 2–4). Interestingly, replacement of the *N*-phenyl group in **10** with an *N*-3-pyridyl group **11** improved potency, indicating that polar amides could be beneficial to the Raf-1 potency.

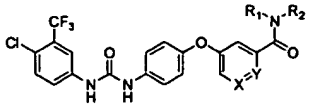
Based on this observation, various polar carboxamides were synthesized; the results are shown in Tables 2–4. In most of these examples, introduction of water-solubilizing groups provided compounds with retained Raf-1 inhibitor potency, and large groups were well tolerated. We hypothesize that this portion of the molecule is directed towards the solvent when the compound is bound to Raf-1 kinase.

Table 1. Substitution of the carboxamide group



Compd	R_1	R_2	Raf-1 kinase IC_{50} (nM)[9]
2	H	Me	120
6	H	Et	130
7	H	*n*-Pr	140
8	Me	Me	5800
9	H	CH_2Ph	460
10	H	Ph	370
11	H	3-Pyridyl	68

Table 2. Carboxamides related to diphenyl urea BAY 43-9006 (4)



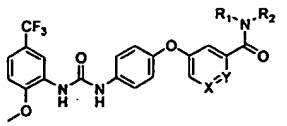
Compd	X	Y	R_1	R_2	IC_{50} (nM)[9]
12	CH	CH	H	Me	130
13	CH	CH	H	4-Morpholinyl-$(CH_2)_2$	70
14	CH	CH	H	1-Piperidinyl-$(CH_2)_2$	82
15	CH	CH	H	2-Et-pyrrolidin-1-yl-(CH_2)	270
16	CH	CH	H	3-Pyridyl	44
17	CH	CH	H	4-(Me_2N)-phenyl	230
18	CH	CH	H	PhNH-$(CH_2)_2$	160
19	CH	CH	H	MeO-$(CH_2)_2$	110
20	CH	CH	H	4-MeO-3-pyridyl	130
21	CH	CH	H	(4-Morpholinyl)phenyl	160
5	CH	N	H	Me	12
22	CH	N	H	Et	26
23	CH	N	Me	Me	300
24	CH	N	H	*i*-Pr	2300
25	CH	N	H	4-Morpholinyl-$(CH_2)_2$	73
26	N	CH	H	4-Morpholinyl-$(CH_2)_2$	140
27	N	CH	H	Me	50

Introduction of a pyridine in place of the distal phenyl ring in such analogues provided compounds with retained high potency (e.g., **25** and **26** vs **13**, Table 2); however, additive effects seen previously in the case of **3** versus **1**, **4** versus **2**, and **33** versus **28** were not realized.

Interestingly, *N*-alkylnicotinamide analogues such as **26–27** and **37–39** (Tables 2 and 3) showed Raf-1 potencies similar to those of the isomeric *N*-alkyl 2-pyridinecarboxamides.

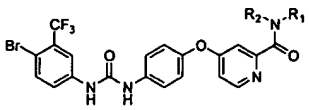
Selected examples were evaluated in an equilibrium-based aqueous solubility assay, according to a high-throughput Nuclear Magnetic Resonance (NMR) flow technology assay protocol developed in-house.[11] The effect of *N*-methyl amide substitution with polar amides on aqueous solubility is shown in Table 5. Compound **39** is more than 10-fold more soluble than the corresponding *N*-methyl amide analogue **38** at physiological pH. As expected from these basic analogues, very good aqueous solubility was observed at lower pH (Table 5), which could potentially provide improved absorption through the gastrointestinal membrane.[11]

Table 3. Carboxamides in the diphenyl urea class



Compd	X	Y	R_1	R_2	IC_{50} (nM)[9]
28	CH	CH	H	Me	130
29	CH	CH	H	3-Pyridyl	100
30	CH	CH	H	4-(Me_2N)-Phenyl	410
31	CH	CH	H	6-MeO-3-Pyridyl	150
32	CH	CH	H	(4-Morpholinyl)phenyl	210
33	CH	N	H	Me	53
34	CH	N	H	Et	460
35	CH	N	Me	Me	330
36	CH	N	H	*i*-Bu	500
37	N	CH	H	4-Morpholinyl-$(CH_2)_2$	63
38	N	CH	H	Me	61
39	N	CH	H	Me_2N-$(CH_2)_2$	100

Table 4. Carboxamides in the diphenyl urea class



Compd	R_1	R_2	IC_{50} (nM)[9]
40	H	Me	6
41	H	Et	27
42	Me	Me	170
43	H	4-Morpholinyl-$(CH_2)_2$	60

Table 5. Aqueous solubility of selected ureas

Compd	Solubility, pH 2.7 (μg/mL)	Solubility, pH 7.2 (μg/mL)
38	≤4	≤4
39	129	41
25	141	≤5
43	39	≤5
26	36	≤5

Conditions: (a) pH 7.2, 0.5×PBS; or pH 2.7, 3 mM citrate, 1.4 mM KCl, 68 mM NaCl; (b) agitate at rt for 3 h; (c) centrifuge and analyze by NMR.[11]

In summary, novel Raf-1 kinase inhibitors from the urea class have been prepared. The carboxamide group of BAY 43-9006 and its analogues was shown to be a suitable position for the introduction of water-solubilizing groups. Improvements of aqueous solubilities by up to 10-fold were realized without significant impact on Raf-1 kinase potency.

Acknowledgements

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References and notes

1. Dumas, J. *Exp. Opin. Ther. Pat.* **2001**, *11*, 405, and references cited therein. Chandra Kumar, S.; Madison, V. *Exp. Opin. Emerg. Drugs* **2001**, *6*, 303.
2. Bridges, A. *Curr. Med. Chem.* **1999**, *6*, 825.
3. Lyons, J. F.; Wilhelm, S. M.; Hibner, B.; Bollag, G. *Endocr.-Relat. Cancer* **2001**, *8*, 219.
4. Smith, R. A.; Barbosa, J.; Blum, C. L.; Bobko, M. A.; Caringal, Y. V.; Dally, R.; Johnson, J. S.; Katz, M. E.; Kennure, N.; Kingery-Wood, J.; Lee, W.; Lowinger, T. B.; Lyons, J.; Marsh, V.; Rogers, D. H.; Swartz, S.; Walling, T.; Wild, H. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2775. Due to slight modifications of the biochemical assay, IC_{50} values obtained during the initial phase of the program were slightly lower than those determined during the later phase, such as described in this report. For example, compound 1 was initially found to have $IC_{50} = 0.54$ μM ($n = 6$).
5. Lowinger, T. B.; Riedl, B.; Dumas, J.; Smith, R. A. *Curr. Pharm. Des.* **2002**, *8*, 99.
6. Riedl, B.; Lowinger, T. B.; Bankston, D.; Barbosa, J.; Brittelli, D. R.; Carlson, R.; Dumas, J.; Hibner, B.; Kadono, H.; Katz, M.: Kennure, N.; Khire, U.; Lee, W.; Monahan, M.-K.; Natero, R.; Renick, J.; Rong, H.; Scott, W. J.; Sibley, R. N.; Smith, R. A., Wood, J. 92nd Meeting of the American Association for Cancer Research, New Orleans, March 24–28, 2001; Abstract 4956.
7. Miller, S.; Osterhout, M.; Dumas, J.; Khire, U.; Lowinger, T.; Riedl, B.; Scott, W.; Smith, R.; Wood, J.; Rodriguez, M.; Wang, M. PCT Int. Appl., WO 99/32436. *Chem. Abstr.* **1999**, *131*, 58658.
8. Riedl, B.; Dumas, J.; Khire, U.; Lowinger, T. B.; Scott, W. J.; Smith, R. A.; Wood, J. E.; Monahan, M.-K.; Natero, R.; Renick, J.; Sibley, R. N. PCT Int. Appl., WO 00/42012. *Chem. Abstr.* **2000**, *133*, 120157.
9. In vitro Raf Kinase activity screen: Human Raf-1 (c-Raf) and Mek were cloned, expressed and purified at Onyx Pharmaceuticals. Briefly, Sf-9 cells were infected with baculovirus encoding epitope-tagged c-Raf or Mek (EYMPME; EE-tag on the C-terminus). Epitope-tagged Raf and Mek were then purified from infected Sf-9 cell lysates by immuno-affinity chromatography, and were stored at −80 °C in the following storage buffers. Raf kinase: 20 mM Tris pH 8.0, 1mM EDTA, 1mM DTT, 20 μM Leupeptin, 1% v/v NP40, 50% glycerol. Mek kinase: 25 mM Tris pH 7.8, 10 mM NaCl, 1 mM EDTA, 1 mM DTT, 4 μM Leupeptin, 50% glycerol. In order to assay for Raf-1 activity, Raf and Mek were diluted together with reaction buffer (200 mM Tris pH 8.2, 100 mM NaCl and 20 mM 2-mercaptoethanol) to 4 and 20 μg/mL, respectively, and 20 μL of this enzyme-substrate mixture was added to each well of a 96-well plate. The kinase reaction was initiated by adding 25 μL of 10 μM γ-[^{33}P]ATP (sp. Act. 400 Ci/mol) for incubation at 32 °C for 25 min. Filtration onto a phosphocellulose mat was used to harvest protein, and 1% phosphoric acid was used to wash away unbound radio-nucleotide. Following drying by microwave heating, the filter was enclosed in a plastic sample bag, scintillation fluid was added, and a b-plate counter was used to measure filter-bound radioactivity. To screen for inhibitors, test compounds were serially diluted from 10 mM stock solutions in DMSO, using a liquid handling robot, into 10% DMSO in water to 10 times the final desired concentrations (1% final DMSO concentration). Five microliters of these serially diluted stocks or matching DMSO containing vehicle was added to the enzyme-substrate mixture, prior to the addition of radiolabelled ATP. IC_{50} values were calculated using a four-parameter non-linear curve-fitting program. At least two independent IC_{50} determinations

were performed on each compound, and the mean value is reported. In all cases, standard deviations were less than 50% of the mean IC_{50} value.

10. For water solubilizing groups we specifically focused on substituents with heteroatoms that could add hydrophilicity or provide a site for salt formation. For a discussion on importance of the aqueous solubility as a drug-like property: Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev.* **2001**, *46*, 3, and references cited therein. Huuskonen, J. *Combin. Chem., High Throughput Screen.* **2001**, *4*, 311.
11. Heald, S. H.; Blake, P. C. *Am. Pharm. Rev.* In press.